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Increased antibacterial activity of plant oils against foodborne pathogens through their encapsulation into chitosan based nanoparticles

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Abstract: Encapsulation of plant oils to increase their antimicrobial properties without losing their bioactive properties is a good strategy. Electrospraying technique is known to be one of the most efficient methods for the encapsulation process for this purpose. In this study, the conditions to fabricate nanoparticles were optimized by considering several characteristics of the nanoparticles such as the particle size using the electrospraying method. A mixture of *Origanum vulgare* L. essential oil and olive oil was encapsulated into a mixed polymer (poly vinyl alcohol/Chitosan; PVA-Chitosan) matrix, and the fabricated nanostructures were characterized. The plant oil loaded nanoparticles (PONPs) were also investigated in terms of their antimicrobial activity against different foodborne bacterial pathogens; namely, *Escherichia coli* ATCC 25150, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 13932 and *Salmonella typhimurium* ATCC 14028. Their antibacterial activities were revealed by the agar disc diffusion method. As a result, PONPs had strong antimicrobial activity against all the tested pathogens. The PONPs showed the highest antibacterial activities against *L. monocytogenes* and *E. coli*, which was revealed by the higher inhibition zone values (15.83 and 11.60 mm, respectively). The results of this study suggest the use of electrospraying technique applied under the optimized conditions in this study as natural carriers to increase antibacterial activity of the plant oils.

Keywords: Increased antimicrobial activity, Nanoparticles, Electrospraying technique, *Origanum vulgare*, Olive oil

1. Introduction

Pathogens are disease causing microorganisms that include bacteria, fungi, parasites, and viruses. Pathogens can cause illness by their transmission to human through spoiled or contaminated food and water. Common symptoms of foodborne illnesses are gastrointestinal problems and vomiting; moreover, some of the more severe cases can result in premature death. Each year, foodborne pathogens such as; *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, and *Toxoplasma gondii* cause an average number changing from 3.3 million to 12.3 million cases of foodborne illness and up to 3.900 cases of deaths (Buzby and Roberts 1996).

Nowadays, the intensive use of antibiotics has led a wide range of foodborne pathogens develop a resistance against the drugs; for that reason, there is a need to research more effective antimicrobial agents (Rodríguez-Tobías et al. 2019). Some compounds from natural sources such as plants have been alternatively proposed as potential antimicrobial agents. Essential oil phenolic substances are mainly responsible for antibiotic properties (Scandorieiro et al. 2016). Oregano is a class of medicinal and aromatic plants and the whole plant is used as an essential oil. Oregano essential oil is of great importance to the pharmaceutical, cosmetic and food industries. Essential oils consist of phytochemicals, monoterpenes, sesquiterpenes and their oxygen derivatives. These phytochemicals (thymol, anethol, menthol, carvacrol, phenolic acids, and flavones, etc.) show antimicrobial activity against bacteria (Yilmaz et al. 2016). Studies on the antibacterial mechanisms of oregano essential oil suggest that hydrophobic bioactive compounds cause damage to cell membrane, affect ATP production and protein synthesis, increase cell permeability, induce cytoplasmic changes and finally cause cell-death (Hyldgaard et al. 2012; Scandorieiro et al. 2016). In this respect, as compared to many other vegetable oils, olive oil has higher content of phenolic compounds and stronger antioxidant properties, which makes it more valuable than other vegetable oils (Sevim et al. 2016). Olive oil is always of special importance because it is a rich source of high

monounsaturated fatty acids (oleic acid) and antioxidants (E-vitamin and phenolic compounds). It is also an important oil in terms of its calcium, magnesium, iron and copper content. In addition, it includes provitamin A, vitamin C and thiamine in sufficient amounts (Yılmaz et al. 2016; Sevim et al. 2016). The content of bioactive components such as chlorophyll, carotenoid, tocopherols and phenolic compounds in olive oil varies according to its type and cultivation conditions, which affects the quality and antioxidant content of olive oil (Sevim et al. 2016).

Recently, nanostructures possessing the well known antimicrobial properties have been used as promising compounds in order to develop nanoparticles showing better antimicrobial performance than traditional antibiotics. For this reason, there is a trend to develop encapsulated essential oils with stronger antimicrobial properties (Rodríguez-Tobías et al. 2019). The influence of nanotechnology particularly on the healthcare industry is also substantial. Nanoparticles are used as natural or synthetic carriers for the delivery of drugs, growth factors, health supplements, and vitamins (Sridhar et al. 2015). There are several techniques that are presently used to develop such carriers, and electrospraying is one of these techniques that have been increasingly used in the recent years (Karakaş et al. 2019). This method can be used for making polymer particles with nanometer scale diameters (Reneker and Chun 1996).

In order to increase the reliability, durability and nutritional value of food products, nanotechnology can be applied to improve the functionality of the bioactive materials by increasing their efficiency in terms of effective distribution of their functional components within the body (Chen et al. 2006a). For this purpose, nano sized food additives have been produced because nano additives are absorbed more easily and increase the shelf life of the product. Nanotechnology has also been used to develop formulation of functional food products. In this regard, food companies are working on the nano delivery system to increase the effectiveness of active ingredients such as vitamins and antioxidants. By using nano delivery systems, it is aimed at increasing control and efficacy of active ingredients (Gomez-Hens and Fernandez-Romero 2006). The food or food components which possess health benefits due to their preventive and therapeutic effects are defined as nutraceuticals or functional foods (Andlauer and Fürst 2002). The efficacy of nutraceuticals in the prevention of diseases depends on the preservation of the bioavailability of the active ingredient. Under normal conditions, the health benefits of the nutraceuticals may be impaired during food processing (light, temperature, and pH) or gastrointestinal system (pH and other nutrients), which leads to the decreased stability of the nutritional elements in food items, their reduced ability to remain in the stomach juice, and the poor permeability or solubility in the intestine. In order for the active ingredient to remain active until it reaches the physiological target in the organism, the ingredient should be in a protected structure in the system (Chen et al. 2006b). In this respect, their encapsulation within nanostructures such as nanoparticles provides the protected structure. Encapsulation within nanoparticles is an efficient technique that offers superior properties than conventional encapsulation methods since the nanoparticles are small in size, which enables the active ingredient to be directly transferred to the circulatory system (Moraru et al. 2003).

Chitosan is a biodegradable and biocompatible biopolymer possessing various promising biological activities such as antibacterial, antifungal and antitumor effects. Hence, there has been a great number of studies to explore its potential applications in various industries. Chitosan has a potential application in several areas such as pharmaceutics, food, biotechnology, and environment (Chung et al. 2004). Chitosan having antimicrobial activity can also be used to extend the storage life of foods by protecting them from attacks of foodborne pathogens. There have been recent extensive efforts to test the antimicrobial capacity of chitosan to protect food (Chung et al. 2003).

This study aims at increasing antibacterial activity of plant oils against foodborne pathogens through their encapsulation into chitosan based nanoparticles. For this purpose, the electrospraying method was used to fabricate nanoparticles to encapsulate a mixture of plant oils (MoPO), namely *Origanum vulgare* L. essential oil and olive oil. In brief, we first prepared this plant oil mixture loaded nanoparticles (PONPs), and then evaluate antibacterial activity of oil loaded nanoparticles against selected bacterial strain.

2. Materials and Method

2.1. Materials

Poly-vinyl alcohol and chitosan were procured from Merck (Merck, Darmstadt, Germany) and Sigma (Sigma-Aldrich, UK), respectively. Tween 20 was used as an emulsifier that was purchased from Sigma (Sigma-Aldrich, UK).Ethanol, methanol, Dimethyl sulfoxide (DMSO) and Nutrient agar (NA) were purchased from Merck (Merck, Darmstadt, Germany). *O. vulgare* was harvested from fields of Çumra Vocational School department of Plantal and Animal Production program of Medical and Aromatic Plants Research Center of Selçuk University, Konya, Turkey. The hydrodistillation method was used to extracted essential oil from *O. vulgare*. Olive oil as the core material was obtained from Tariş, Turkey. In all experiments, deionization water was used.

2.2. Electrospraying Method

PVA-chitosan solution was prepared as follows, 0.4 gr PVA was dissolved in 24.5 mL distilled water and 0.1 gr chitosan was dissolved in 0.5 mL acetic acid at 70°C for 4 hours by stirring on a magnetic stirrer. After that, 1% (w/v) of the MoPO (mixture of 200 µL olive oil and 10 µL *O. vulgare*) and 1% (w/v) of Tween 20 were added and mixed to obtain a homogeneous solution (Fig.1). Holmarc Opto Mechatronics Pvt. Ltd. Nano Fiber Electrospinning Unit model no: HO-NFES-0434 electrospinning apparatus was used to fabricate mixture of plant oil nanoparticles (PONPs) by spraying method. The nanoparticles were

collected on a 10 cm collector plate at a rate of 0.3 mL/h. The electrical current was adjusted to 3 mA and the voltage to 19.8-20 kV (Karakaş et al. 2019).

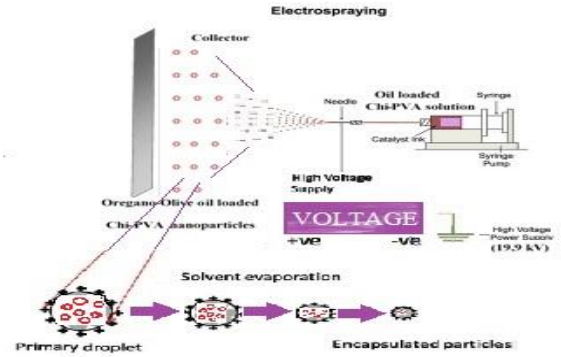

Fig. 1 The electrospraying system and experimental layout

2.3. Characterization of Nanoparticles

Particle diameters and polydispersity of PONPs were measured using a Zetasizer (Malvern Nano ZSP instrument, England). Quartz cuvettes were used during measurement. The surface morphology was revealed by images of scanning electron microscopy (SEM, Zeiss EVO ls-1, Germany) at a magnification of 25.00 K ×. The molecular structure was identified by FTIR (Thermoscientific Bruker Alpha model, Germany) to prove encapsulation.

2.4. Antibacterial Properties of Nanoparticles

Agar disk diffusion method was used to perform in vitro susceptibility tests for *Escherichia coli* ATCC 25150, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 13932 and *Salmonella typhimurium* ATCC 14028 strains. A blank disc impregnated with the 30µl volume of PONPs at different concentrations was placed on the agar plates to enable the antimicrobial substances to diffuse from the disc into the agar. The PONPs were prepared in DMSO at 10 mg mL^{-1} concentration (Vehapi et al. 2018a). After that, the plate was inoculated with a standardized suspension of a microorganism and incubated at 37 ˚C for 24 h. After the incubation period, the growth inhibition zones around the discs were measured (Sasidharan et al. 2010; Vehapi et al. 2018b). The diameter of the zone indicated the antimicrobial susceptibility of the test microorganism (Figure 2). The concentration of culture suspensions was adjusted to 106 CFU/mL by PG Instruments T-60 Uv Visible Spectrophotometer device at 600 nm.

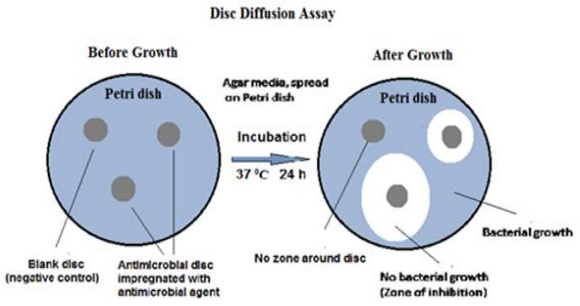

Fig. 2 A schematic design of agar disk diffusion assay

2.5. Statistical Methods

Analysis of variance was performed using the JMP (release 6.0.0, SAS) package program. The significance ratings between the averages were determined by Student's t test using the same package program. Data were presented as means with ± standard deviations (Correa et al. 2011; Natalie et al. 2017).

3. Results and Discussion

3.1. Characterization of Electrosprayed Nanoparticles (PONPs)

The diameters of the particles fabricated by the electrospray technique was investigated at different parameters. Particle diameters were measured using a Zetasizer instrument (Table 1). The particles were fabricated according to optimized parameters; namely, certain flow rate of the polymer solution, levels of voltage and current, and distance between the injection needle and the collector plate.

Table 1 Particle size and zeta potentials of PONPs.

Sample	Z-Ave	PDI	PK2 Mean Int. d:nm	PK2 Mean Int. d:nm	PK1 Area Int. percent	PK2 Area Int. percent
1	1196	0.94	428.7	72.52	86.6	13.4
2	1131	0.76	400.0	45.91	91.0	9.0

Morphology of blank NP0 and PONPs (Figure 3) were observed by SEM analysis. The PONPs were fabricated using the PVA-chitosan polymer solution. The average particle size of PONPs was determined to be 594.2 nm and the average particle size of blank nanoparticles was about 360 nm (Figure 3). It was observed that PONPs had higher particle size than did blank nanoparticles. Plant oil loaded nanoparticles PONPs were resulted in spherical shapes, and smooth surface structure.

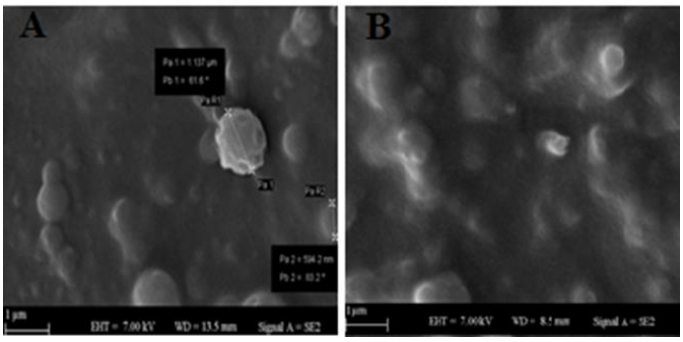

Fig. 3 SEM images of PONPs (A) and blank NP0 (B) nanoparticles at magnification of 25.00 Kx

The functional groups of PONPs were revealed by the FTIR analysis. The absorbance values of the functional groups present in the samples were determined in the wavelengths from 4000 to 600 cm^{-1} (Figure 4). The FTIR spectra revealed characteristic peaks for C-H tension at

2800-2900 cm^{-1}, alkene tension at 1600-1700 cm^{-1}, C = O ester bond tension at 1700 cm^{-1} and ester C-O tension and C-N stretching at 1000-1200 cm^{-1}. These characteristic peaks were compared with those of PVA-Chitosan polymer solution and plant oil mixture.

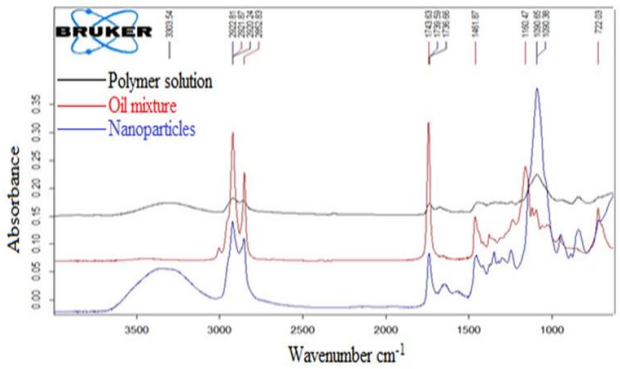

Fig. 4 FT-IR spectra of PONPs, plant oil mixture and polymer solution

The spectrum of PONPs was different from the spectra of plant oil mixture and polymer solution in terms of additional peaks belonging to plant oils, which indicated the oil mixture could be successfully encapsulated within the nanoparticles by electrospray technique.

3.1. Antibacterial Activity Against Foodborne Pathogens

Table 2 shows the size of inhibition zones (mm) formed against *E.coli*, *B. cereus*, *L. monocytogenes*, and *S. typhimurium* strains.

Table 2 Size of inhibition zone (mm) of PONPs, polymer solution, free plant oil and DMSO against foodborne pathogens

Sample	*E.coli*	*L.monocytogenes*	*B.cereus*	*S.typhi*
PONPs	$11.6 \pm 0.4^{A,b}$	$15.8 \pm 0.7^{A,a}$	$9.5 \pm 0.7^{A,c}$	$9.0 \pm 0.7^{A,c}$
PVA-Chi	$9.0 \pm 0.0^{B,a}$	$9.0 \pm 0.0^{B,a}$	$8.0 \pm 0.0^{B,b}$	$8.0 \pm 0.0^{B,b}$
MoPO	$9.0 \pm 0.0^{B,a}$	$9.0 \pm 0.0^{B,a}$	$8.0 \pm 0.0^{B,b}$	$8.0 \pm 0.0^{B,b}$
DMSO	$7.0 \pm 0.0^{C,a}$	No Zone	$7.0 \pm 0.0^{C,a}$	No Zone

* Data were presented as means ± standard deviations(n=6) ($p < 0.05$). MoPO: Mixture of plant oils

A–C: Within each column, different superscript uppercase letters show differences between the treatments.

a–c: Within each row, different superscript lowercase letters show differences between the bacterial strains.

The disc diffusion assay results showed that PONPs exhibited stronger antibacterial activity against *L. monocytogenes* than other treatments. The size of inhibition zone formed by PONPs against *L. monocytogenes* was 15.83±0.76 mm. On the other hand, these nanoparticles showed similar or lower antibacterial activity against *B. cereus* and *S. typhimurium*. The size of inhibition zone formed by PONPs against *B. cereus* and *S. typhimurium* were 9.50±0.70 mm and 9.00±0.70 mm, respectively. The size of inhibition zone formed by PONPs against *E. coli* was 11.60±0.45 mm (Table 2). From Table 2, it is clear that free oil had lower antibacterial effects than the encapsulated oil, revealing that the antimicrobial effectiveness of the plant oils could be increased by their encapsulation within the polymeric nanoparticles using the electrospraying technique.

4. Conclusion

Chitosan, essential oil and nanotechnology interactions may be potential strategies for controlling resistance evolution of foodborne pathogens. Our results highlight the powerful action of the plant oils encapsulated within PVA-chitosan polymer mix by electrospraying technique against Gram-negative and Gram-positive bacterial strains. In conclusion, the encapsulated plant oils resulted in synergistic and additive antimicrobial activities against foodborne pathogens such as *E. coli*, *B. cereus*, *L. monocytogenes* and *S. typhimurium*. Therefore, the results of this study suggest that PONPs (plant oil loaded nanoparticles) would have a potential applications in cosmetics, food and pharmaceutical industry as well as clinical and hospital settings; i.e., for treating foodborne infections.

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AZ: contributed to the supply of oils and the electrospraying process of nanoparticles.

MV: contributed to the characterization of nanoparticles and antimicrobial activity test.

Conflict of interest disclosure:

There is no conflict of interest.

References

Andlauer W, Fürst P. 2002. Nutraceuticals: a piece of history, present status and outlook. Food Res Int 35: 171-176.

Buzby JC, Roberts T. 1996. ERS updates US foodborne disease costs for seven pathogens. Food Rev/Natl Food Rev 19(1482-2016-121439): 20-25.

Chen H, Weiss J, Shahidi F. 2006a. Nanotechnology in nutraceuticals and functioanal foods. Food Technol 60 (3): 30-36.

Chen L, Remondetto GE, Subirade M. 2006b. Food protein-based materials as nutraceutical delivery systems. Trends in Food Sci Technol 17(5): 272-283.

Chung YC, Su YP, Chen CC, Jia G, Wang HL, Wu JG, Lin JG. 2004. Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacol Sin 25(7): 932-936.

Chung YC, Wang HL, Chen YM, Li SL. 2003. Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. Bioresour Technol 88(3): 179-184.

Correa H, Aristizabal F, Duque C, Kerr R. 2011. Cytotoxic and antimicrobial activity of pseudopterosins and seco-Pseudopterosins isolated from the octocoral Pseudopterogorgia elisabethae of San Andrés and Providencia Islands (Southwest Caribbean Sea). Mar Drugs 9: 334-344.

Gomez-Hens A, Fernandez-Romero JM. 2006. Analytical methods for the control of liposomal deliver system. TrAC Trends Anal Chem 25: 167-178.

Hyldgaard M, Mygind T, Meyer RL. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 3:12. doi: 10.3389/fmicb.2012.00012

Karakas CY, Sahin HT, lnan B, Özçimen D, Erginer YÖ. 2019. In vitro cytotoxic activity of microalgal extracts loaded nano–micro particles produced via electrospraying and microemulsion methods. Biotechnol Progress e 2876: 1-2. https://doi.org/10.1002/btpr.2876.

Moraru C I, Panchapakesan C P, Huang Q, Takhistov P, Liu S, Kokini JL. 2003. Nanotechnology: A new frontier in food science understanding the special properties of materials of nanometer size will allow food scientists to design new, healthier, tastier, and safer foods. Nanotechnol 57(12): 24-29.

Natalie PB, Bobbi LH, James JG. 2017. Endophytic root bacteria associated with the natural vegetation growing at the hydrocarbon-contaminated Bitumount Provincial Historic site. Can J Microbiol 63(6): 502-515. dx.doi.org/10.1139/cjm-2017-0039

Reneker DH, Chun I. 1996. Nanometre diameter fibres of polymer, produced by electrospinning. Nanotechnol 7(3) : 216.

Rodríguez-Tobías H, Morales G, Grande D. 2019. Comprehensive review on electrospinning techniques as versatile approaches toward antimicrobial biopolymeric composite fibers. Mater Sci Eng: C 101:306-322

Sasidharan S, Darah I, Noordin MKMJ. 2010. In vitro antimicrobial activity against *Pseudomonas aeruginosa* and acute oral toxicity of marine algae *Gracilaria changii*. New biotechnol 27(4): 390-396

Sevim D, Köseoğlu O, Çetin Ö. 2016. Bazı önemli zeytin çeşitlerinden elde edilen yağların minör bileşenlerinin ve antioksidan aktivitesinin belirlenmesi. Zeytin Bilimi 6 (1): 1-7.

Scandorieiro S, de Camargo L C, Lancheros CA, Yamada-Ogatta SF, Nakamura C V, de Oliveira AG, ... & Kobayashi RK. 2016. Synergistic and additive effect of oregano essential oil and biological silver nanoparticles against multidrug-resistant bacterial strains. Front microbial 7: 760.

Sridhar R, Lakshminarayanan R, Madhaiyan K, Barathi VA, Chin Lim KH, Ramakrishna S. 2015. Electrosprayed nanoparticles and electrospun nanofibers based on natural materials: applications in tissue regeneration, drug delivery and pharmaceuticals. Chem Soc Rev 44(3): 790-814.

Vehapi M, Yılmaz A, Özcimen D. 2018a. Investigation of antibacterial and antioxidant activities of some algae species. J Biotechnol 280: S80.

Vehapi M, Yılmaz A, Özcimen D. 2018b. Antifungal activities of *Chlorella vulgaris* and *Chlorella minutissima* microalgae cultivated in bold basal medium, wastewater and extract water against *Aspergillus niger* and *Fusarium oxysporum*. Rom Biotechnol Lett 1-8.

Yilmaz A, Ermis E, Boyraz N. 2016. Investigation of in vitro and in vivo anti-fungal activities of different plant essential oils against postharvest apple rot deseases *Colletotrichum gleosporioides, Botrytis cinerea* and *Penicillium expansum*. J Food Saf Food Qual 67: 113-148.